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Influence of material extracted from Diploposthe laevis (Bloch) and D. bifaria (Siebold in Creplin) (Cestoda) on tapeworm Hymenolepis diminuta (Rudolphi) in rats

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ABSTRACT. Interspecific relationships in tapeworms were investigated. Acetone-extracted oil (AEO) and post-acetone water extracts (WE) were prepared from the worms Diploposthe laevis (Bloch, 1782) and D. bifaria (Siebold in Creplin, 1846), collected from the ducks Aythya ferina (L.) and A. nyroca (Güld.) These materials were intragastrically administered to Wistar rats, which had earlier been experimentally infected with the tapeworm Hymenolepis diminuta (Rudolphi, 1819). The rats were sacrificed and the behaviour of H. diminuta in the intestine was observed in order to determine its reaction to the worm extracts. For comparison, the behaviour of H. diminuta in infected, bat untreated, rats was examined. It was found that AEO exerted a toxic effect on H. diminuta, whereas WE showed no activity. Analogous materials prepared from random species of tapeworms collected from the duck genus Anas had no effect on H. diminuta.

According to studies on the duck genus Aythya (SULGOSTOWSKA, 1976, 1977), several parasite species occur in the intestine of young A. ferina (L.) and A. nyroca (Güld.) as early as in other ducks. However, if by any chance in this intestine the tapeworms Diploposthe laevis (Bloch, 1782) or D. bifaria (Siebold in Creplin, 1846) are present, then with the development of the host only one of these species becomes dominant (in A. ferina mainly D. laevis and in A. nyroca mainly D. bifaria). Thus there is an elimination of other parasite populations, as well as of the "superfluous" individuals of its own population, when only two or occasionally three individuals are left. The ecological causes of this phenomenon include the ability of the population of these tapeworms to dominate the worm community. This paper presents some experimental results which indicate that biochemical factors may also be involved in this phenomenon.

MATERIALS AND METHODS

Preparation of acetone-extracted oil (AEO) from tapeworms D. laevis and D. bifaria (collected in Poland and in Hungary).

The abovementioned tapeworms were isolated from the alimentary tract of A. ferina and A. nyroca, washed from intestinal contents with water and placed in vials with about 5 ml of acetone. This material was kept at room temperature for 4 months. After completion of this extraction period, the tapeworms were removed, rinsed with pure acetone, and dried to constant weight in a dessicator under vacuum, at room temperature. The combined acetone extract and washings were evaporated under vacuum at 40°C, yielding a pale-yellow oil. This oil was dissolved in 50 ml of anhydrous ethanol; ethanol was evaporated and the oil was dried in a dessicator under vacuum, over P_2O_5. The oil was soluble in ethanol and chloroform,

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* Part of the preparation of this work was done by the senior author during her visit to Hungary in 1983.

whereas it was insoluble in water. Heating of the oil with water up to 40°C, with intense stirring, yielded an emulsion stable for several hours. The emulsion was extracted four times with 100 ml of chloroform. The combined chloroform extract was liberated from chloroform by evaporation of the oil which was dried in a dessicator under vacuum. This procedure provided a yield of oil of about 10% against the dry weight of tapeworms.

For comparison, the material comprising random species of tapeworms collected from duck Anas genus was subjected to an identical treatment.

Preparation of post-acetone water extract (WE) from the tapeworms D. laevis and D. bifaria

Tapeworms previously extracted with acetone were homogenized with a 10-fold volume of water in a glass homogenizer. The solid parts were removed by centrifugation, and volumes of the supernatant corresponding to 500 mg dry weight of the tapeworms were taken for the biological activity tests.

WE was also prepared from random tapeworm species.

Biological tests

Hundred Wistar rats (1 year old) were experimentally infected with 4-5 Hymenolepis diminuta (Rud., 1819) larvae per rat. Four weeks after the experimental infection, rat faeces were examined for the presence of H. diminuta.

In the biological tests the aqueous emulsion of AEO and WE from D. laevis and D. bifaria as well as from the random tapeworms, were administered intragastrically to the rats with a probe.

Table 1. Effectivity of tapeworm extracts on Hymenolepis diminuta population in rat

Experimental groups	30 rats treated with AEO_1	30 rats treated with WE_1	15 rats treated with AEO_2	15 rats treated with WE_2	10 rats control
Results	H. diminuta ejected, rejected, compactly curled	no effect	slightly effected	no effect	H. diminuta uneffected
Effective %	100 %	0 %	20 %	0 %	-

AEO_1 = Acetone extracted oil from Diploposthe laevis and D. bifaria
WE_1 = Water " " " " " "
AEO_2 = Acetone extracted oil from mixed tapeworms of duck
WE_2 = Water " " " " " " "

After definite periods the rats were sacrificed and dissected in order to determine the reaction of tapeworm H. diminuta to the investigated materials.

The behaviour of H. diminuta tapeworms in infected rats (10 specimens) which were not treated with the investigated materials served as control.

RESULTS

In the first experiments the dose of the AEO_1 emulsion required to exert a toxic action on H. diminuta was determined. The applied doses of AEO_1 corresponded to 125, 250, 500 and 800 mg of dry weight of D. laevis and D. bifaria. It was found that a perceptible toxic effect was exerted by a dose of AEO_1 corresponding to 500 mg dry weight of tapeworms; this amount could be obtained from 4-7 tapeworms (Table 1).

The toxic effect on H. diminuta manifested itself in the following five ways: 1) translocation of tapeworm from the anterior of the jejunum to its further parts, and even to the

ileum; 2) in all experiments, rejection of the posterior segments of strobila, which were found in the caeca, rectum and faeces; 3) distinct lowering of tapeworm viability; 4) in many cases the tapeworms were compactly curled up, whereas in untreated rats they rested loosely along the jejunum; 5) in some cases the oncospheres originating from the rejected segments optically appeared to be damaged.

Essential changes in the behaviour of H. diminuta took place during the first 2-3 hrs after the administration of the studied materials.

Experiments on characterization of the active substance (s) from isolated D. laevis and D. bifaria showed the presence of activity in AEO; a lack of activity in WE and the absence of activity in the aqueous phase after chloroform extraction of AEO.

AEO from other tapeworm species, parasites of the duck genus Anas showed, very slight toxic activity. No activity was found in WE extracted from other tapeworms.

These results testify to the non-polar lipophylic nature of the substance (s) obtained from D. laevis and D. bifaria which are active against H. diminuta.

DISCUSSION

So far no conclusive concept interpreting the interrelations between parasites in hosts has been put forward. Some authors emphasise the importance of ecological factors and consider the problem from the standpoint of population structure; others stress the role of chemical factors, and yet others confine themselve to the presentation of the experimental results, without interpreting the underlying mechanisms.

In the literature some data concerning the reactions of one parasite species to other parasites are reported. For example, LARSH and DONALDSON (1964) have studied the interspecific relationship in the populations of the nematode Nippostrongylus brasiliensis (Travassos, 1914) = N. muris (Yokogawa, 1920) and the tapeworm Hymenolepis nana (Siebold, 1882). According to these authors, the nematodes adversely affect the development of tapeworms, because in the case of a mixed invasion only less than one half of the tapeworms remained in the intestine, as compared with a single-species invasion. PAWLOWSKIJ and GNIEZDILOW (1950) have found that in dogs infected with the nematode Toxocara canis (Werner, 1782) fewer plerocercoides of the tapeworm Diphyllobothrium latum (L., 1758) remained alive; mean body weight and length of the tapeworms were much smaller as compared with tapeworms in dogs free from nematodes. In studies on interspecific relationships in populations of the nematodes Trichuris muris (Schrank, 1788) and Aspiculuris tetraptera (Nitzsch, 1821), KEELING (1961) has demonstrated the occurrence of an antagonism between these parasite species; the antagonism was shown by a reduction of the number of parasites of both species during a mixed invasion. The latter author has also found that the sequence of introduction of the parasite species influenced the number of settled individuals of the second parasite; in these studies T. muris proved to be more sensitive to the presence of A. tetraptera invasion.

In our study we have found that D. laevis and D. bifaria contain substances which can exert a toxic effect on other Cestoda species. They are extracted by acetons or chloroform which indicates their lipid nature.

The present results suggest that the secretion of active chemical substances by parasites may regulate the numbers of other parasites in the host. The action of these compounds could explain for instance the exclusive occurrence of either D. laevis or D. bifaria - in a number of only 1-2 individuals - in the duck genus Aythya. Moreover, the results of studies on the effectiveness of the material from tapeworms parasitizing ducks against tapeworms occurring in rats seem to suggest that the toxic action of the investigated materials is non-specific.

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